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ADDITION OF EXTRACTIVES OF ROTENONE-BEARING PLANTS TO SPRAY OILS[1, 2]

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INTRODUCTION

ALTHOUGH THE addition of extractives[7] of rotenone-bearing plants to spray oils used against scale insects on citrus trees has long occupied the attention of entomologists (Ebeling, 1940),[8] it is only recently that oils to which toxicants have been added were used extensively in commercial practice in southern California. Commercial use of oils plus toxicants has had the effect of emphasizing practical problems such as (1) the possibility of dissolving adequate concentrations of extractives in spray oils without the use of a mutual solvent; (2) the passage of a toxicant from the oil to the water phase of an emulsion; (3) the effect of the different types of mutual solvents used to incorporate the extractives into the spray oil, on the physical nature and stability of the toxic solution and on the insecticidal effectiveness of the spray; (4) the effect of the mutual solvents on the oil-depositing properties of the spray; (5) decomposition of the toxicant during the varying periods between manufacture and use; (6) the relative value of rotenone, rotenone-free extractives, and total extractives of rotenone-bearing plants; and (7) the long-term effect on the scale population density resulting from the lighter oils which are made more effective by the addition of a toxicant, but which nevertheless do not leave a long-lasting film of oil on the tree to retard the development of the progeny of those scales which fail to succumb to the oil. The purpose of this paper is to evaluate the relative importance of these and other incidental factors and to suggest means by which present difficulties may be overcome.

SOLUBILITY OF DERRIS OR CUBÉ EXTRACTIVES IN OIL

Solubility of Derris or Cubé Extractives in Oil without the Aid of a Mutual Solvent.—The prevailing preoccupation of entomologists with mutual solvents as a means of incorporating derris and cubé extractives into oil should not

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[7] The total resinous material extracted from derris or cubé root by organic solvents and containing the insecticidal ingredients of the root.

[8] See "Literature Cited" at end of the paper for complete data on citations, which are referred to in the text by author and date of publication.

direct attention away from the fact that a considerable concentration of extractives can be dissolved directly in the oil even at room temperature. Two grams of derris extractives (35 per cent rotenone) which had been powdered and sieved through an 80-mesh screen was placed in each of two 500 ml flasks; 100 ml of light-medium straight oil[9] was added to one flask, and 100 ml of light-medium emulsive[10] oil was added to the other. Both flasks were then tightly corked and shaken by hand continuously and uniformly for 20 minutes at 25° C. The oils were then filtered through a Seitz pad (no. 6), and the concentration of rotenone plus deguelin in the filtrates was determined according to Gunther's modification of the Gross and Smith color test (Gunther and Turrell, 1944). The concentration of rotenone plus deguelin was 0.8 gram per liter in the straight oil, and 1.5 grams per liter in the emulsive oil.

The experiment was later repeated with derris extractives of a different origin, which had been extracted with a different solvent and contained only 30 per cent rotenone. Four grams of the powdered extractives (80-mesh) was added to 100 ml of straight oil and to 100 ml of emulsive oil, both in 500-ml flasks as before. The two flasks were placed in a shaking machine constructed for the purpose to obviate any possibility of error due to a difference in the degree of agitation of the liquid. The concentration of rotenone plus deguelin was 1 gram per liter in the straight oil, and 2 grams per liter in the emulsive oil. Still higher concentrations of extractives can be obtained by the use of heat.

The glyceryl dioleate in the emulsive oil made it possible in both tests to approximately double the amount of rotenone plus deguelin dissolved. Glyceryl dioleate is surface-active, as is shown by the fact that it lowers interfacial tension, and it is a good solvent for derris or cubé extractives, so that at the interface between the oil and the resin particle it apparently acts as a solvent or solubilizer to bring the rotenone plus deguelin more rapidly into solution in the oil. English (1939) greatly increased the efficiency of an oil-derris mixture when he used diglycol oleate as an emulsifier, but he used this solute at such a high concentration in the oil (10 per cent) that the solute itself might have been the carrier of the toxic ingredients of the derris powder.

It is interesting to note, as will be shown presently, that, in the case of the straight oil, about as high a concentration of rotenone plus deguelin can be obtained by soaking finely ground cubé root in the oil as was obtained by soaking the extractives in oil, and this concentration can be obtained in just as short a period as when the total derris extractives are dissolved. It should be borne in mind, however, that the extractives were from different plants; *Derris* in one case and *Lonchocarpus* in the other.

To determine the amount of rotenone plus deguelin that can be dissolved in oil from cubé root (5 per cent rotenone) ground so that not less than 95 per cent passed through a 200-mesh screen, various amounts of the ground root ranging from ½ to 12 grams were added to 100-ml portions of light-medium spray oil in 500-ml flasks. The flasks were then continuously shaken by hand in a uniform manner, as before, for 20-minute periods at 25° C. The oil was

[9] Fifty-six per cent distilled at 636° F; viscosity, 70 seconds Saybolt at 100° F; unsulfonatable residue 90 per cent. A straight oil is one to which no solute has been added.

[10] An emulsive spray oil is one which contains usually about 1 per cent of an oil-soluble emulsifier, which greatly reduces the interfacial tension between oil and water. The emulsive oil used in the experiments presented in this paper contained 1 per cent glyceryl dioleate.

then filtered through a Seitz pad (no. 6) and the concentration of rotenone plus deguelin in the filtrate was determined in the same manner as for the previous filtrate. The concentrations of rotenone plus deguelin in the filtrates are shown in figure 1. Curve *B* shows the increase in concentration of rotenone plus deguelin with increasing amounts of cubé root added to straight light-medium oil, and it also shows the decreasing *rate* of solution.

Curve *A* in figure 1 indicates the far greater efficiency obtained in the extraction of rotenone plus deguelin from ground cubé root if emulsive spray oil

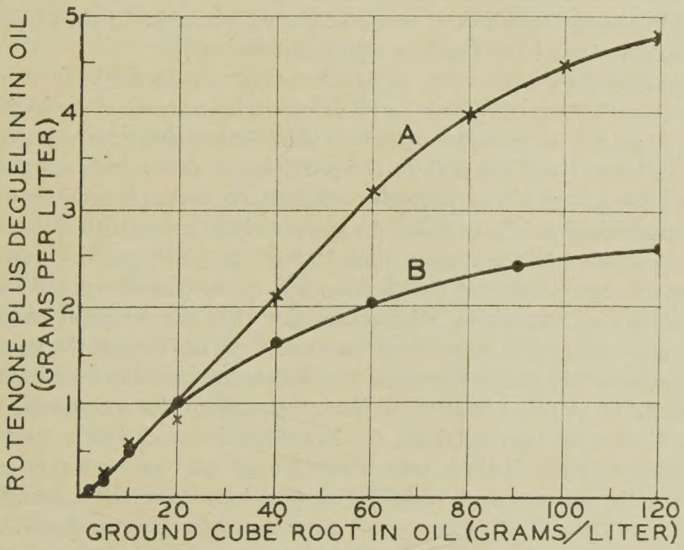

Fig. 1.—The increase in the amount of rotenone plus deguelin extracted from various amounts of finely ground cubé root (5 per cent rotenone) shaken in 100-ml portions of emulsive and straight spray oils for 20-minute periods. *A*, Emulsive oil; *B*, straight oil.

is used as the solvent when the amount of the ground root is greater than 20 grams per liter of oil. The superiority of the emulsive oil above this point becomes relatively greater as the proportion of the ground root is increased; when 120 grams of ground root per liter is used the emulsive oil is almost twice as effective a solvent as the straight oil. The two oils are identical except for the 1 per cent glyceryl dioleate in the emulsive oil.

The experiment was repeated, but with a cubé root of a lower rotenone-plus-deguelin content. This time the flasks containing the oil and ground root were shaken together on a shaking machine for 20 minutes. Although the amount of rotenone plus deguelin dissolved by the oil was lower than in the previous experiment, the superiority of the emulsive oil in extracting these compounds was just as great as before.

In two separate tests, when 40 and 60 grams of ground cubé root were shaken in a shaking machine in 1 liter of emulsive oil for 1 hour, the amount of rotenone plus deguelin extracted was no greater than when the same amount of root was shaken in the oil for only 20 minutes.

Emulsive oils would probably differ in their solvent properties according to

the nature of the solute they contain. Likewise the nature of the ground root would also have an effect on the amount of rotenone plus deguelin dissolved by the oil. In the present investigation the ground root of *Derris* containing 6 per cent rotenone and ground to a 200-mesh-particle size was shaken in emulsive oil for 20-minute periods in order to compare the amount of rotenone plus deguelin extracted by emulsive oil from derris root with that which had been extracted from cubé root (*Lonchocarpus*) containing 5 per cent rotenone under identical conditions. The derris root was added to the emulsive oil at the rate of 20, 40, 80, and 120 grams per liter. The average concentration of rotenone plus deguelin in the oil was 36.2 per cent less than that which resulted from the extraction of the same amounts of cubé root.

In comparing the solubility in oil of the rotenone plus deguelin as contained in the total derris extractives and as it exists in its natural state in the ground cubé root, it should be borne in mind that the root particles were on the average probably more finely divided than the particles of derris extractives, since 95 per cent of the ground root particles would pass through a 200-mesh screen, while the powdered derris extractives were sieved through an 80-mesh screen. However, since probably not more than 10 per cent of the ground root material will respond to the Gross and Smith color test as compared with at least 60 per cent of the derris extractives, it is remarkable that the rotenone plus deguelin can be so easily extracted from the ground root. In the case of the straight oil, a higher concentration of the rotenone plus deguelin was obtained in 20 minutes from 2 grams of ground root than from 2 grams of the powdered derris extractives. This may be explained by investigations of derris root made by Gunther and Turrell (1944), who showed that the whole derris extractives were present in the root as a colloidal suspension of particles ranging in size from 0.77μ to 3.85μ in diameter, and were concentrated in parenchymatous extractive-bearing cells, which may be more completely disintegrated in the grinding process than the rest of the root cells. These particles of extractives as they occur in nature are extremely small in comparison with those resulting from powdering of the total extractives with mortar and pestle, which may account for the ease with which they may be dissolved. The over-all effect of the grinding of derris root is that each particle of ground root (cellulose and extraneous matter) becomes coated with a thin film of the much smaller particles of whole derris extractives. Rapidity of solution is an inverse function of particle size.

However, it has long been recognized that the maximum concentration of derris or cubé extractives in oil may, for practical purposes, be best obtained by means of mutual solvents. The latter may be divided into two groups: solubilizers and oleotropic solvents.

True Solubilizers.—Some mutual solvents form a colloidal solution of the extractives in the oil, and these will be referred to as *solubilizers*, their effect being different from that of the mutual solvents which form molecular solutions. The term "solubilizer" may be found in entomological literature as referring to a solvent of any type which can bring into solution in oil a substance ordinarily not oil-soluble. However, McBain and his associates (1941) have considered solubilization as referring only to the preparation of thermodynamically stable colloidal solutions by the addition of otherwise insoluble

materials to dilute aqueous or nonaqueous solutions of detergents. They state, "We may venture the conclusion that it is possible to solubilize almost any material in almost any solvent, as desired. A good solubilizer should be effective in concentrations of 1 per cent or less."

These authors (1941) distinguished between solubilization and the change in solvent power when large quantities of a second solvent are added to a liquid which is by itself a poor solvent. They also show the difference between solubilization and emulsification, suspension, or peptization. Further, they cite the X-ray evidence of Kiessig and Philippoff (1939) on the nature of the lamellar micelles enclosing benzene solubilized in sodium oleate solutions; and they contribute three other distinct types of experimental evidence, produced in their own laboratories, to support the conclusion that solubilization consists in the spontaneous stable formation of self-organized colloidal particles.

To demonstrate the colloidal nature of toxic solutions containing Cardolite[11] as a solubilizer, test tubes were filled with unit quantities of a light-medium spray oil containing various concentrations of the standard Cardolite-extractives-oil stock solution (see footnote 11). A 250-watt, 12-volt electric light bulb was enclosed in a box with an 11-inch square base and 13 inches high, which was lined on the inside with tin foil. A ⅜-inch circular hole was drilled into the top of the box directly above the light bulb, in order that a beam of light might be cast upward from the box into a dark room. The test tubes containing the solutions were placed in a vertical position above the hole. The light from the bulb passed through the center of the column of liquid, producing a Tyndall effect which proved the presence of discrete particles in the solution. The liquid surrounding the beam of light was also somewhat luminous. If oil alone is examined in the above manner, it will be found to show only a faint path of bluish fluorescence.

An attempt was made to determine whether rotenone alone would form a colloidal solution when dissolved in Cardolite at a concentration of 16⅔ per cent and added to spray oil. However, the Cardolite would dissolve not more than 4 per cent of rotenone, and when this solution was diluted with oil in any proportion, one was formed which showed no Tyndall effect. This was also true when only 4 per cent total derris extractives was dissolved in the Cardolite. It may be that the rotenone in an oil-Cardolite-extractives solution is in true solution and that the colloidal particles are made up of the remaining rotenone-free extractives. However, if only 4 per cent of rotenone or total extractives were dissolved in the Cardolite in the preparation of a stock solution, too much of the latter would have to be added to the oil in order to obtain an adequate concentration of the toxicant to substantially increase its effectiveness in

[11] Cardolite 627 is a condensation polymer of formaldehyde and the phenolic constituents of cashew-nut-shell oil (cardanol). A stock solution was prepared which consisted of 4.16 per cent derris extractives (30 per cent rotenone), 20.84 per cent Cardolite 627, and 75 per cent light-medium oil; all percentages by weight. The solution contains 1.25 per cent, by weight, of rotenone. The derris extract is added to the Cardolite at a temperature of 125° C and is dissolved in 1 or 2 minutes, then immediately further diluted with oil to reduce the temperature of the solution below that which is considered detrimental to rotenone.

At the time of this investigation the writers knew of no method for the determination of rotenone in solutions containing Cardolite. Therefore, a method was devised which involved a chromatographic treatment of the toxic solution in order that rotenone might be separated and subsequently determined colorimetrically (Gunther, 1942).

citrus-pest control. Such high concentrations of Cardolite in the oil adversely affect emulsification in the spray tank.

When Cardolite-extractives-oil solutions containing a concentration of rotenone plus deguelin of from 0.04 to 0.20 gram per 100 ml are filtered through a Seitz pad (no. 6) their concentrations of rotenone plus deguelin may be reduced as much as 50 per cent, many of the larger colloidal particles being removed. The luminosity of the path of light passing through a column of the filtrate is much less than that observed in the unfiltered solution, and the liquid surrounding the path of light is nearly "optically void," having largely lost the luminosity observable in unfiltered solutions. A filtrate with a concentration of 0.12 gram per 100 ml of rotenone plus deguelin was found to show less Tyndall effect than an unfiltered solution with a concentration of rotenone plus deguelin of only 0.04 gram per 100 ml. This again indicates that a portion of the extractives in the Cardolite-extractives-oil solution is in molecular solution.

Oleotropic Solvents.—A different type of solution is obtained when solvents not having the ability to solubilize are added in large quantities to a less effective solvent, with the result that the mixture will dissolve substances which would otherwise not go into solution. This is a far less efficient method of getting a substance into solution than is solubilization. A large quantity of a good solvent is necessary in order to make a poor solvent effective; and conversely a small amount of poor solvent will spoil a good solvent. However, if a solution it obtained it is a molecular solution rather than one predominantly colloidal, such as is obtained by solubilization.

Neuberg (1916) proposed the name "hydrotropic" for solvents which, when added to water in large amounts, will increase the solubility of a substance not ordinarily water-soluble. Twenty five per cent or more of the hydrotropic solvent is ordinarily added. The term "hydrotropic" would be a misnomer when applied to solvents added to oil. The writers therefore propose the term *oleotropic* as descriptive of solvents other than solubilizers that may be added to oil to increase its ability to dissolve substances ordinarily insoluble in oil.

The oleotropic solvents which have been tried at this station with more or less success were for the most part esters, ketones, and a complex hydroxy ether, 2 (4-tertiary butylphenoxy) ethanol. When 5 per cent derris extractives (30 per cent rotenone) is dissolved in *n*-butyl phthalate, 1 part of the resulting solution must be added to 2 parts of spray oil in order to obtain a clear solution of the extractives. The same concentration of derris extractives dissolved in diamyl phenol may be diluted 1 to 20 in spray oil and still result in a clear solution. Diamyl phenol is the most effective oleotropic solvent the present writers have investigated, but unfortunately it is somewhat injurious to citrus foliage. A 10 per cent solution of derris extractives in 2 (4-tertiary butylphenoxy) ethanol resulted in a slightly cloudy solution at the dilution of 1 part to 7 parts of oil, but formed a clear solution at higher concentrations in the oil. Using Cardolite as a solubilizer, however, a 16⅔ per cent solution of derris extractives has been diluted in the ratio of 1 part to 156 parts of oil in commercial spraying practice; it can be diluted much more than this and still maintain a visually clear colloidal solution, which, however, shows a Tyndall effect at any concentration in the oil.

PASSAGE OF THE TOXICANT FROM THE OIL TO THE WATER PHASE OF AN EMULSION

Theoretical Considerations.—The possibility of the loss of toxicity due to the passage of derris or cubé extractives from the oil to the water phase of a spray mixture has been a matter of concern among those working with oil-toxicant sprays. A theoretical consideration of the problem, however, indicates that loss of toxicity from this source could, at the most, be only extremely small if the extractives are in true monomolecular solution in the oil.

When a substance has reached an equilibrium between two immiscible solvents the ratio of the concentrations in the two phases will have a constant value at a given temperature:

$$\frac{C_1}{C_2} = K \qquad (1)$$

When a petroleum oil solution containing rotenone is agitated with water, the rotenone passes into the aqueous phase until the equilibrium ratio characteristic of the substances in question is established. When the solute is sparingly soluble, as in the system under consideration, this ratio is identical with the ratio of the solubilities of the substance in each solvent. If there are several solutes in solution, the distribution of each is the same as if it were present alone. A more formal treatment of this principle leads to the equation (Robertson, 1938):

$$x = \frac{K\, v_1\, a}{v_2 + K\, v_1} \qquad (2)$$

when x is the weight of rotenone passing from the oil to the water, K is the equilibrium ratio and is equal to $\frac{S_1}{S_2}$ when S_1 is the solubility of the rotenone in the water and S_2 is the solubility of the rotenone in the oil, a is the original weight of rotenone, v_1 is the volume of water, and v_2 is the volume of petroleum oil. This equation is applicable to the system under consideration because of the extremely small percentages of the solutes involved.

Frear (1942) states that the solubility of rotenone in water is about 160 micrograms per liter at 25° C. The present writers, by adding a large excess of powdered rotenone to light-medium spray oil at 100° C and keeping the oil, at this temperature, stirred for 10 hours by means of an electric mixer, then cooling to 25° C, found the solubility of rotenone in the oil to be about 1.6 grams per liter at 25° C.

Converting these solubilities to the same units, at 25° C,

$$K = \frac{S_1}{S_2} = 1 \times 10^{-4}\,.$$

Assume that 1 gram of rotenone is dissolved in 1 liter of the oil and that the resulting solution is mixed with 49 liters of water at 25° C, as in a 2 per cent oil-toxicant spray, until equilibrium has been attained. In order to determine

the weight of rotenone (x) which has entered the aqueous phase, the appropriate values are substituted into formula 2:

$$x = \frac{(10^{-4})\ (49)\ (1)}{(1) + (10^{-4})\ (49)} = 4.8 \times 10^{-3} \text{ gram of rotenone in the 49 liters of aqueous phase.}$$

Thus 0.48 per cent of the original quantity of rotenone dissolved in the oil has been transferred from that phase to the aqueous phase.

Experimental Results.—It can be seen from the above calculation that theoretically, when dealing with the very dilute solutions of field practice, the amount of rotenone leaving the oil and entering the water phase of an emulsion as a solute is of no practical importance. Experimental evidence substantiated this conclusion. Derris extractives containing 35 per cent rotenone were dissolved in light-medium oil with the aid of heat. A 1¾ per cent concentration of the toxic oil was agitated in 5 gallons of water in a 10-gallon-capacity spray tank for 45 minutes. A part of the spray mixture was then sprayed into a liter graduate, and a gram of powdered calcium chloride was added to the spray mixture to aid in the separation of the oil and water. In 5 minutes the oil which had risen to the surface was transferred to a test tube and centrifuged for 15 minutes to separate the oil completely from the small amount of residual water. A portion of the original oil sample which had not been mixed with the water also was centrifuged. According to an analysis for rotenone plus deguelin made by means of Gunther's modification of the Gross and Smith color test, the oil which had not been mixed with water had a concentration of rotenone plus deguelin of 0.0080 gram per 100 ml, while the oil which had been vigorously agitated with water for 45 minutes had a concentration of rotenone plus deguelin of 0.0079 gram per 100 ml, which did not indicate a significant loss of the toxicant from the oil phase. Under identical conditions an emulsive oil solution containing a concentration of rotenone plus deguelin of 0.20 gram per 100 ml of oil had this concentration reduced to 0.19 gram per 100 ml of oil by agitation with water for 45 minutes; this again indicates a very small loss, if any, of the rotenone plus deguelin to the water phase of the spray mixture.

A test which was performed in the same way, but in which the toxic oil contained 1 part of a Cardolite-derris-extractives-oil solution (see footnote 11) to 19 parts of light-medium spray oil, revealed a loss of about 25 per cent of the rotenone plus deguelin from the oil phase of the spray mixture. It is not known what proportion of the extractives lost was rotenone and what proportion was deguelin. From a concentration of rotenone plus deguelin of 0.04 gram per 100 ml in the original oil, the concentration was reduced to 0.03 gram per 100 ml. In another experiment with the Cardolite-extractives-oil solution, the reduction in concentration of rotenone plus deguelin due to a 45-minute agitation with water was from 0.72 gram per 100 ml to 0.48 gram per 100 ml, representing a loss of one third of the toxicant.

It appears that although rotenone and deguelin in molecular solution in oil do not pass from the oil to the water phase to any appreciable extent, a solution which is largely colloidal may lose as much as a third of its content of toxic ingredients which respond to the Gross and Smith test, probably by *particle* exchange.

EFFECT OF THE MUTUAL SOLVENT ON OIL DEPOSIT AND ON THE INSECTICIDAL PROPERTIES OF THE TOXIC SOLUTION

Despite the greater efficiency of Cardolite as a mutual solvent, extensive investigation has indicated that the oleotropic solvents are usually more effective as far as insecticidal properties of the oil-toxicant solution in which they are contained are concerned (Ebeling, 1940). However, when very low concentrations of derris or cubé extractives are sufficient for the required insecticidal effect, as in the kerosene-toxicant sprays to be described in this paper, Cardolite appears to be the best and most practical mutual solvent of all those which the writers were able to test.

Smith (1932) seems to have been the first to use *n*-butyl phthalate as a mutual solvent in the preparation of oil-rotenone solutions. The high degree of effectiveness of oils containing derris extractives with *n*-butyl phthalate as an oleotropic solvent was shown in field experiments made at this station several years ago (Ebeling, 1940). Two per cent medium tank-mix oil spray resulted in a 21.2 per cent survival of adult female California red scale on the branches of lemon trees, and a 14.4 per cent survival on the fruit. When 1 part of *n*-butyl phthalate containing 5 per cent derris extractives (34 per cent rotenone) was added to 7 parts of medium oil and this oil-toxicant solution was used at 2 per cent, the per cent survival on the branches and fruit was, respectively, 3.9 per cent and 2.5 per cent (Ebeling, 1940).

Cressman (1941) also demonstrated the high degree of effectiveness against red scale of toxic solutions of cubé extractives (30 per cent rotenone) in oil with *n*-butyl phthalate as the mutual solvent. He stated, however, that a *n*-butyl phthalate-trichloroethylene mixture was more efficient as a solvent than *n*-butyl phthalate alone.

When Cardolite 627 was first tried as a means of incorporating derris or cubé extractives into spray oil, high hopes were held for the material because of its extreme efficiency; less than ½ per cent concentration of the solubilizer in oil was sufficient for the solubilization of practical quantities of derris extractives. Inspection of the three-component phase diagrams worked out by Kagy and Boyce (1941) shows that Cardolite was the only solvent which could bring any appreciable amount of derris extractives into solution in oil at concentrations of less than 5 per cent of solvent. However, some solvents were more efficient than Cardolite at higher concentrations of solvent. This may be explained by the fact that true solubilization requires only very small amounts of the solubilizer, and that which is added above this critical amount is superfluous and will not result in correspondingly greater incorporation of the extractives in the oil.

Combined with this high degree of solubilizing efficiency was the added incentive to the use of Cardolite caused by the fact that no other solvent could be used successfully with kerosene; such high concentrations of the kerosene were used in citrus spraying that oleotropic solvents would have been impractical, as well as being injurious to foliage, because of the large quantities which would have been necessary to bring about a solution of the extractives in the kerosene. Moreover, the kerosene-toxicant solutions, using Cardolite as a solubilizer, were highly effective against the red scale when freshly prepared solutions were used at a 10 per cent concentration (Ebeling, 1941).

A factor which excludes from consideration some otherwise excellent mutual solvents is excessive emulsifying ability of the solvent or excessive spreading of the spray mixture caused by the solvent. This results in too greatly reduced oil deposit with a consequent reduction in the effectiveness of the oil spray which may outweigh the advantages gained from the introduction of an effective toxicant. An example of such a solvent is 2 (4-tertiary butylphenoxy) ethanol, which was one of the most efficient of the oleotropic solvents tested, yet has such great emulsifying and spreading properties that it cannot be successfully used against insects requiring a heavy oil deposit for successful control, such as the red scale. Against other scales, such as the black scale, *Saissetia oleae* (Bernard), the successful control of which does not require a heavy film of oil, if the oil is sufficiently toxic, the 2 (4-tertiary butylphenoxy) ethanol may be used very successfully (Boyce and associates, 1940).

TABLE 1

DIFFERENCE IN EFFECTIVENESS AGAINST CALIFORNIA RED SCALE OF SPRAYS CONTAINING SOLUTIONS OF DERRIS OR CUBÉ EXTRACTIVES IN LIGHT-MEDIUM EMULSIVE SPRAY OIL, USING OLEOTROPIC SOLVENTS, A SOLUBILIZER, AND NO MUTUAL SOLVENT*

Treatment no.	Material	Concentration of extractives in the oil (per cent by weight)†	Oil deposit on leaves (ml/cm²)10⁶		Net per cent survival‡	
			Orchard A	Orchard B	Orchard A	Orchard B
1	Oil without toxicant	....	100.3	102.3	25.1	40.2
2	Oleotropic solvent: *n*-butyl phthalate with 5 per cent derris extractives, 1 part to 15 parts of oil	0.31	....	85.8	....	9.1
3	Oleotropic solvent: *n*-butyl phthalate with 5 per cent derris extractives, 1 part to 7 parts of oil	0.62	68.7	79.3	5.6	0.9
4	Oleotropic solvent: *n*-butyl phthalate with 10 per cent derris extractives, 1 part to 15 parts of oil	0.62	62.6	....	19.2	....
5	Oleotropic solvent: diamyl phenol with 10 per cent derris extractives, 1 part to 15 parts of oil	0.62	....	85.0	....	2.3
6	Solubilizer: oil-Cardolite with 4.16 per cent derris extractives, 1 part to 15 parts of oil	0.26	78.3	93.8	29.3	9.9
7	Solubilizer: oil-Cardolite with 4.16 per cent derris extractives, 1 part to 7 parts of oil	0.52	76.2	....	24.6	....
8	No mutual solvent: ground cubé root, 4 ounces to 1 gallon of oil	0.14	76.5	83.4	9.9	8.8
	Least significant difference for odds of 19 to 1	....	....	....	8.4	6.3

* The oil used was 54 per cent distilled at 636° F; viscosity, 69 seconds Saybolt at 100° F; unsulfonatable residue, 90 per cent. In all treatments the oil or oil-toxicant was used at 2 per cent concentration. The treatments in orchard A were made on April 9, 1942, and those in orchard B were made on July 6, 1942, and in each grove the scales were examined about 6 weeks after treatment.

† The percentages of extractives in this and following tables are calculated, except in treatments in which the ground cubé root was soaked in the oil. In the latter the values were determined by analysis and do not show the per cent of extractives other than rotenone and deguelin.

‡ From 4,354 to 8,763 adult female red scales were examined for each treatment. In the entire experiment, 80,399 insects were examined. Variation between trees was determined in each plot and subsequently pooled for all plots. This served to provide a standard error for mean values of each plot, hence for standard errors of difference between any two plots. From this the least significant difference as based on Fisher's t test was calculated.

Field Tests with Oil-Toxicant Solutions Containing Different Mutual Solvents.—To further test the greater insecticidal effectiveness of oil-toxicant solutions containing oleotropic solvents as compared with those containing

true solubilizers, some field experiments were designed to make possible a direct comparison of the two types of solvents within a single orchard. In one experiment (table 1, orchard A) the sprays were applied to an orange orchard and in the other (table 1, orchard B) they were applied to a lemon orchard, both orchards being heavily infested with California red scale. The sprays were applied with a high-pressure power sprayer with $^{8}/_{64}$-inch disks in the nozzles, and with a degree of thoroughness comparable to the average commercial work in citrus spraying in southern California.

From 5 to 6 weeks after treatment the adult scales were examined *in situ* to determine the percentage surviving treatment. The interim between spraying and examination was sufficient to insure the death of those insects not immediately killed by the spray and to permit the complete desiccation of the dead scales to facilitate accurate and rapid determination as to whether the scales were alive or dead.

In each plot, 10 trees were examined and on each of these trees the number of insects on 20 units of branches (a unit consisting of from 4 to 6 inches of the branch as seen from one aspect) were recorded, together with the number alive. An average of about 6,000 scales per plot were counted. The net per cent survival was determined by dividing the percentage found alive in the treated plots by the percentage found alive on 10 untreated trees, examined at the time the counts were made on the treated plots, and multiplying the quotient by 100. Separate tabulations of the net per cent survival were made for each tree so that an estimate could be made of the error within plots.

Immediately after spraying, 100 orange leaves or 75 lemon leaves were picked and placed in quart jars to be later subjected to a steam-distillation treatment (Gunther and Ebeling, 1942) for the determination of the amount of oil applied per unit of leaf surface. The oil deposit per square centimeter is shown in tables 1, 2, 3, and 4.

Table 1 shows the great difference in insecticidal effectiveness of a given concentration of derris extractives in emulsive spray oil depending on the kind of mutual solvent used. Although in this particular experiment the concentration of derris extractives in the Cardolite solutions was a little less than in the *n*-butyl phthalate solutions (0.26 and 0.52 per cent by weight as compared with 0.31 and 0.62 per cent in the latter), it has been shown (Ebeling and LaDue, 1943) that if the concentration of derris extractives is twice that shown in table 1, with a corresponding increase in Cardolite, the effectiveness of the oil spray is not significantly increased. Perhaps this is because straight oil will not emulsify properly if too much Cardolite is added. When emulsive oils are used, however, this difficulty is overcome, but the insecticidal effectiveness of the oil is still not improved by increased concentrations of the Cardolite-extractives solution.

Table 2 shows the results of an experiment in a lemon orchard on August 26 and 27, 1942, in which the Cardolite-extractives solution was used with both emulsive and straight oil of the light-medium grade. Although there was a substantial improvement in kill due to the toxicant when straight oil was used, there was no improvement with emulsive oil, even though the same concentration of toxicant was the same in each treatment.

It will be noted (table 1, orchard A) that when an *n*-butyl phthalate partial

solution containing 5 per cent derris extractives was used at 1 part to 7 parts of oil, the concentration of extractives was the same as when 10 per cent derris extractives was used at 1 part to 15 parts of oil, yet the former resulted in significantly better kill of red scale. The superiority of the 5 per cent partial solution is apparently due to the greater degree of solution; the liquid was only slightly cloudy as compared with the very cloudy appearance of the 10

TABLE 2

THE EFFECTIVENESS OF DERRIS EXTRACTIVES IN SPRAY OIL AS SHOWN BY TWO IDENTICAL SERIES OF EXPERIMENTS IN A LEMON ORCHARD*

Treatment no.	Material	Extractives in oil (per cent by weight)	Oil deposit on leaves (ml/cm²)10⁶	Net per cent survival†	
				Series A	Series B
1	Heavy-medium emulsive oil	....	97.9	41.4	40.4
2	Light-medium emulsive oil	....	90.8	46.0	50.4
3	Light-medium emulsive oil; oil-Cardolite with 4.16 per cent extractives, 1 part to 13 parts of oil	0.31	107.9	41.0	38.4
4	Light-medium straight oil; oil-Cardolite with 4.16 per cent extractives, 1 part to 13 parts of oil	0.31	105.9	23.3	26.2
5	Light-medium straight oil; oil-Cardolite with 4.16 per cent extractives, 2 parts to 13 parts of oil	0.62	109.6	26.2	25.8
6	Light-medium emulsive oil; *n*-butyl phthalate with 5 per cent extractives, 1 part to 7 parts of oil	0.62	81.6	16.5	15.2
7	Same as no. 6 except that the *n*-butyl phthalate-extractives solution was 54 days old	0.62	79.5	15.5	20.2
8	Light-medium emulsive oil; *n*-butyl phthalate with 3.75 per cent extractives, 1 part to 7 parts of oil	0.46	75.7	19.0	27.1
9	Light-medium emulsive oil; *n*-butyl phthalate with 5 per cent extractives, 1 part to 15 parts of oil	0.31	80.8	31.2	34.2
10	Light-medium emulsive oil; ground cubé root, ½ pound to 2 gallons of oil	0.12	83.9	17.5	25.6
11	Light-medium emulsive oil; ground cubé root, 1 pound to 2 gallons of oil	0.17	86.2	17.9	24.9
12	Light-medium emulsive oil;‡ *n*-butyl phthalate with 5 per cent extractives, 1 part to 7 parts of oil	0.62	63.6	50.1	51.4
	Least significant difference for odds of 19 to 1	....	....	10.1	9.5

* The plots in series A were treated August 26, and those in series B, August 27, 1942. All oils or oil toxicants were used at 2 per cent concentration except in treatment 12, in which 1.5 per cent was used.

† From 4,954 to 6,602 adult female red scales were examined in each plot. The entire experiment involved the examination of 129,881 scales. Variation between trees was determined in each plot and subsequently pooled for all plots. This served to provide a standard error for mean values of each plot, hence for standard errors of difference between any two plots. From this the least significant difference as based on Fisher's t test was calculated.

‡ The oil toxicant in plot 12 was used at 1.5 per cent concentration in the spray mixture.

per cent partial solution when the two were added to the spray oil. Apparently, with any given mutual solvent, the greater the per cent of extractives actually in solution, the greater the insecticidal efficiency of the toxic solution. Presumably the suspended material does not readily penetrate the armor or body wall of the scale.

It can be seen from table 1, orchard B, treatment 5, that diamyl phenol may be successfully used as an oleotropic solvent. This is in accord with the investigations of Kagy and Boyce (1941), who found diamyl phenol to be more effective as a mutual solvent, on the basis of plait-point determinations. than *n*-butyl phthalate. Unfortunately, some injury to citrus foliage results from the use of diamyl phenol.

The entire series of treatments listed in table 2 was repeated in the same orchard in order to determine how closely the net percentages of survival in the various plots would coincide in the two series of tests. These are recorded in the table as series A and series B. Although in this experiment a considerable percentage of insects survived in all the treatments, it should be borne in mind that the effectiveness of an oil spray is in part due to its residual effect in inhibiting the settling of "crawlers" produced by those scales which do not succumb to the spray treatment. The final insecticidal effectiveness of an oil spray is therefore greater than that which is indicated by per cent kill.

Again the oil toxicant containing a calculated concentration of derris extractives of 0.625 per cent (table 2, treatments 6 and 7) was found to be the most effective spray oil despite the lowering of oil deposit on the leaf surfaces due to the presence of the *n*-butyl phthalate in the spray mixture. However, in series A the oil containing ground cubé root (treatments 10 and 11) was not significantly inferior even though there was a much lower concentration of extractives in the oil. Treatment 7 shows that a 54-day period did not result in a significant reduction in the effectiveness of the *n*-butyl phthalate-extractives solution, as indicated by the percentages of survival for this treatment in both series of tests.

As in table 1, treatment 8, the finely ground cubé root (200-mesh, and containing 5 per cent rotenone) was stirred in the oil for about a minute; then the mixture was allowed to settle for about 20 minutes—the minimum period required to empty the average 400-gallon spray rig.

In both table 1 and table 2 it can be seen that although the cubé root did not result on the average in so great an improvement in the spray oil as that which resulted from a 5 per cent concentration of derris extractives in *n*-butyl phthalate used at a strength of 1 part to 7 parts of oil, its effectiveness per unit of extractives in the oil was considerably greater. The explanation probably is the fact that the extractives incorporated into the oil by means of *n*-butyl phthalate are partly in suspension, while those extracted by the oil are entirely in solution. If the ground root had been agitated in the oil in the same manner as the samples represented in figure 1, the percentages of rotenone plus deguelin in the oil would have been 0.15 per cent in treatment 10, and 0.32 per cent in treatment 11 (table 2), according to curve *A* in figure 1. The fact that the percentages were actually much lower (0.12 per cent and 0.17 per cent, respectively) indicates that the field method of mixing the root used in treatments 10 and 11 should be improved in order to bring about better extraction of the toxic ingredients.

To determine to what extent the extraction of rotenone plus deguelin from cubé root can be improved by proper mixing, the equivalent of 1 pound of ground cubé root (5 per cent rotenone) in 2 gallons of light-medium emulsive oil was stirred in a beaker for 1 minute and allowed to stand for 19 minutes. The concentration of rotenone plus deguelin in the oil, at the end of the 20-minute period, was 0.19 per cent by weight. When the ground root and oil were stirred violently with an electric mixer for 1 minute and allowed to stand 19 minutes, the concentration of rotenone plus deguelin in the oil was 0.25 per cent. When the ground root and oil were stirred to a froth by means of an electric mixer for 20 minutes, the concentration of rotenone plus deguelin in

the oil was 0.35 per cent. It appears, therefore, that greater effectiveness of the ground cubé root and oil mixture than that which is indicated in tables 1 and 2 might be obtained if the mixture were stirred continuously for 20 minutes instead of 1 minute, as was done in the experiments recorded in this paper. However, extraction of the toxic constituents of the ground cubé root

TABLE 3

EFFECTIVENESS OF CARDOLITE AS A SOLUBILIZER FOR DERRIS EXTRACTIVES IN KEROSENE*

Treatment no.	Material	Kerosene deposit on leaves (ml/cm²)10⁶	Net per cent survival†
	Series A; sprayed April 9, 1942		
1	Kerosene without toxicant	457.1	49.9
2	One part of freshly prepared oil-Cardolite-extractives solution to 39 parts of kerosene‡	572.4	1.8
3	One part of kerosene-Cardolite-extractives solution prepared 6 weeks prior to use, to 39 parts of kerosene	503.9	20.2
4	One pound of ground cubé root (200-mesh, 5 per cent rotenone) to 20 gallons of kerosene	419.4	4.9
	Series B; sprayed May 25, 1942		
5	One part of freshly prepared kerosene-Cardolite-extractives solution to 39 parts of kerosene	576.5	2.9
6	One pound of ground cubé root (200-mesh, 5 per cent rotenone) to 20 gallons of kerosene	318.5	1.7
7	One pound of ground devil's shoestring, *Tephrosia virginiana* (200-mesh, approximately 1.5 per cent rotenone) to 10 gallons of kerosene	475.9	10.9
	Least significant difference for odds of 19 to 1	......	8.4

* Initial boiling point 360° F; end point 498° F, unsulfonatable residue 95 per cent plus. The kerosene, or kerosene plus toxic solution, was used in all plots at 10 per cent concentration with 4 ounces of calcium caseinate spreader to 100 gallons of spray.

† From 3,576 to 6,291 adult female red scales were examined in each plot. The treatments represented in this table were a part of the series shown in table 1, orchard A, and the least significant difference was calculated coincidently with that of the data shown in table 1.

‡ The kerosene-Cardolite-extractives solution contains 75 per cent kerosene, 20.84 per cent Cardolite, and 4.16 per cent derris extractives (30 per cent rotenone).

continues to take place after the oil is placed in the spray tank if complete extraction has not been attained before (Ebeling and LaDue, 1943).

It can be seen from table 2, treatment 11, that a reduction in the amount of oil deposited on the tree overcomes the advantage resulting from the addition of the toxic solution and gives no better kill of red scale than that obtained from 2 per cent oil without a toxicant.

Tests with Kerosene-Toxicant.—The high degree of effectiveness of freshly prepared kerosene-Cardolite-extractives solution, when used at 10 per cent concentration in the control of the red scale (table 3), may appear paradoxical in view of the relatively far less satisfactory results obtained when Cardolite is used as a solubilizer with the regular spray oils. However, as has already been shown, a certain percentage of the extractives in a Cardolite-extractives-oil solution appears to be in molecular rather than in colloidal solution. Sometimes only a low per cent of extractives in the oil is necessary in order to obtain

the desired insecticidal effect, as is the case when 10 per cent toxic kerosene spray is used against the red scale. In such cases, Cardolite affords an efficient means of preparing the toxic solution, for only a very small amount is required to incorporate the extractives into the oil as compared with the oleotropic solvents with which the writers have experimented.

The ability that Cardolite has of causing the beginning of an inversion of phases, or what Knight (1942) calls "unstable equilibrium" conducive to maximum oil deposit, may be one of the factors favoring its use in the kerosene spray; this increase in deposit is shown in table 3. Sometimes the Cardolite will even cause a doubling of oil deposit on the foliage of citrus trees when added to kerosene in the concentrations shown in table 3.

The amount of rotenone and deguelin extracted by kerosene when 1 pound of ground cubé root (95 per cent passing through a 200-mesh screen and containing 5 per cent rotenone) is added to 20 gallons of kerosene, stirred with a paddle, and allowed to stand for 20 minutes, is equivalent to that dissolved in the oil when 2 quarts of toxic Cardolite solution containing 4.16 per cent total derris extractives and 1.25 per cent rotenone is added to 19½ gallons of kerosene, namely, 0.05 gram per 100 ml. The two types of kerosene-toxicant spray have also, on the average, been about equally effective.

The kerosene-toxicant spray is discussed in this paper because of its academic interest only. The possibility of injury to the bark of citrus trees below the soil line if the kerosene is allowed to soak into the ground immediately adjacent to the trunk of the tree, and the occasional "gumming" of the trunk and branches of lemon trees, stand in the way of commercial use of kerosene sprays at 10 per cent concentration on citrus trees.

DECOMPOSITION OF THE TOXICANT

The practicability of using finely ground cubé root with spray oil has been demonstrated. The oil-toxicant sprays containing only the ground root have the advantage of costing less per unit of rotenone plus deguelin dissolved in the oil, since the cost of extraction and preparation of a toxic stock solution is saved. Also the ground root can be stored for years without appreciable decomposition. Martin (1942) found no reduction in per cent of total extractives, rotenone, or rotenone plus deguelin when air-dried ground derris root was stored in tins for a period of from four to six years. On the other hand the rotenone-plus-deguelin content of an oil-Cardolite-extractives solution is reduced 24.4 per cent during the first month and is entirely decomposed at the end of 8 months (Gunther, 1943). This decomposition is largely eliminated by the addition of 1½ per cent by weight of hydroquinone. This indicates that the decomposition is largely an oxidation phenomenon, although since the pH of an aqueous solution of Cardolite 627 is 8.35, the basicity of the toxic stock solution accounts for some degree of decomposition (see Cahn, Phipers, and Boam, 1938*a*).

The effect of a 6-weeks' period of decomposition unimpeded by antioxidants is shown in table 3 by the great difference in net per cent survival of adult red scale in the plot in which a freshly prepared kerosene-Cardolite-extractives solution was added to the kerosene, as compared with an adjoining plot in which a 6-week-old solution containing the same proportions of ingredients

prepared in the same manner was used. The old solution was taken from a commercially prepared solution which had been stored in a 30-gallon drum on a spray-rig tender on which it had frequently been exposed to the direct rays of the sun. Probably many of the unsatisfactory results which have been reported in the commercial use of kerosene-toxicant spray in certain sections of southern California during the 1941 spraying season were caused by delay in the use of the toxic solution after its preparation. The results shown in table 3 indicate to what extent decomposition may unfavorably influence the results of an oil-toxicant spray. The decomposition of rotenone and deguelin in an oil-Cardolite-extractives solution is much more rapid than in *n*-butyl phthalate. As has already been shown (table 2), no significant reduction in the insecticidal effectiveness of a solution of *n*-butyl phthalate extractives could be demonstrated after a 54-day period.

THE APPARENT SYNERGISM OF ROTENONE AND ROTENONE-FREE EXTRACTIVES

Haller, Goodhue, and Jones (1942) have pointed out that although earlier work had indicated that rotenone was the most toxic of the extractives of derris root against certain insects, later work by H. A. Jones and associates had shown that powdered derris extractives containing about 25 per cent of rotenone were as toxic to mosquito larvae as was pure rotenone; also that F. L. Campbell *et al.* had found that a kerosene extract of derris from which no rotenone could be extracted was effective against houseflies. Bliss (1939) showed evidence of synergism of recrystallized rotenone and the "dehydro mixture" in the residual resin after the removal of the rotenone, in an evaluation of data from the experiments of Tattersfield and Martin (1935) on the toxicity of derris extractives to *Aphis rumicis*.

In table 4 are presented the results of a test to demonstrate the effect of (1) rotenone alone, (2) the rotenone-free extractives remaining when the rotenone is removed from the derris extractives (sometimes referred to as "deguelin concentrate"), and (3) the entire derris extractives (30 per cent rotenone) when used in regular oil spray and in kerosene oil spray, directed against the red scale. The rotenone was removed from the complete derris extractives by means of fractional crystallization of a carbon tetrachloride solution of the extractives. When used with 2 per cent light-medium spray oil, the toxicants were dissolved in *n*-butyl phthalate (table 4, plots 2, 3, and 4) and when used with 10 per cent kerosene spray the toxicants were dissolved in Cardolite (table 4, plots 6, 7, and 8), for reasons which have already been discussed.

In the light-medium spray oil, the same percentage of toxicant was used, whether it was rotenone, rotenone-free resins, or complete derris extractives. This was impossible when the kerosene-Cardolite solutions were used because it was impossible to maintain as high a percentage of rotenone in solution in the kerosene as of complete extractives. Therefore only as much rotenone was added as is ordinarily dissolved in the kerosene when the total extractives are added (1.25 per cent of the stock kerosene-Cardolite-extractives solution or 0.031 per cent of the finished kerosene-toxicant solution). Likewise as much of the rotenone-free extractives was added as is ordinarily dissolved in the kero-

sene when the total extractives are added (2.9 per cent of the stock solution or 0.072 per cent of the finished kerosene-toxicant solution). The usual toxic stock solution, which contains 4.16 per cent total derris extractives, was then added to kerosene at the rate of 1 quart to 9¾ gallons of kerosene. The finished toxic solution then contained 0.103 per cent total extractives, which is the sum of the per cent of rotenone in the solution used in treatment 6 and the per cent of rotenone-free extractives in the solution in treatment 7.

TABLE 4

RELATIVE EFFECTIVENESS OF ROTENONE, ROTENONE-FREE RESINS, AND COMPLETE DERRIS EXTRACTIVES IN SPRAY OIL AND KEROSENE*

Treatment no.	Material†	Concentration of extractives in the oil (per cent by weight)	Oil deposit on leaves $(ml/cm^2)10^6$	Net per cent survival‡
1	One part of *n*-butyl phthalate to 7 parts of oil (no toxicant)	.....	66.5	27.3
2	One part of a 5 per cent solution of rotenone in *n*-butyl phthalate to 7 parts of oil	0.62	57.8	10.7
3	One part of a 5 per cent solution of rotenone-free resins in *n*-butyl phthalate to 7 parts of oil	0.62	62.7	13.5
4	One part of a 5 per cent solution of total derris extractives to 7 parts of oil	0.62	68.7	5.6
5	Kerosene alone (no toxicant)	.....	457.1	49.9
6	One part of a kerosene-Cardolite-extractives solution (with 1.25 per cent rotenone) to 39 parts of kerosene	0.031	554.0	5.1
7	One part of a kerosene-Cardolite-extractives) solution (with 2.9 per cent rotenone-free resins) to 39 parts of kerosene	0.072	573.3	11.0
8	One part of kerosene-Cardolite-extractives solution (with 4.16 per cent total derris extractives) to 39 parts of kerosene	0.103	572.4	1.8
	Least significant difference for odds of 19 to 1	.....	.....	8.4

* Specifications for spray oil and kerosene; and dates of treatment and examination, same as in tables 1 and 2.

† The spray oil or spray oil plus toxicant was used at 2 per cent concentration, and the kerosene or kerosene plus toxicant was used at 10 per cent concentration. No spreader was used with the spray oil, since it was of the emulsive type, and 4 ounces calcium caseinate spreader to 100 gallons of spray was used with the kerosene.

‡ From 4,354 to 8,763 adult female red scales were examined in each plot. The treatments represented in this table were a part of the series shown in table 1, orchard A, and the least significant difference was calculated coincidently with that of the data shown in table 1.

The percentages of survival of red scales in treatments 2 and 3 (table 4) were not significantly different, indicating that in regular spray oil the rotenone-free extractives are approximately as effective as a similar concentration of rotenone when used against the red scale. Both toxicants result in a statistically significant increase in the effectiveness of the oil when present at a concentration of 0.62 per cent in the oil. A 0.62 per cent concentration of the complete extractives, however, resulted in a 5.6 per cent survival of red scale, which was significantly less than the per cent survival in treatments 2 and 3. The experiment indicates, first, that the rotenone-free extractives have a demonstrable toxic effect when added to spray oil by means of a suitable mutual solvent, which appears to be approximately equal to that of rotenone; and, secondly, that the combined toxic effect of rotenone and the rotenone-free extractives of derris extractives is greater than would be predicted from ex-

periments with the isolated constituents. In other words, there appears to be a synergistic effect when the different toxic ingredients of derris extractives are used together in their normal proportions. Another field test[12] not recorded in this paper has led to the same conclusion.

The 0.031 per cent solution of rotenone in kerosene resulted in a lower per cent survival (table 4) than the 0.072 per cent solution of rotenone-free extractives in kerosene, although again both solutions resulted in highly significant increase in the effectiveness of the oil. The total extractives at a 0.103 per cent concentration were more effective than the contained rotenone or rotenone-free extractives when used separately; but in this case no synergistic effect can be inferred because the per cent of total extractives was greater than the per cent of either the rotenone or the rotenone-free extractives. Unfortunately, a 0.103 per cent concentration of rotenone could not be used with the kerosene-Cardolite solution because that concentration of rotenone does not remain in solution.

COMPARISON OF TREATMENTS AT LONG PERIODS AFTER SPRAYING

Comparisons of treatments on the basis of per cent mortality or per cent survival immediately after treatment, or, as in the case of the red scale, a month after treatment, have been criticized on the basis that the relative effectiveness of treatments 6 months or a year after the application of the spray might be different from the apparent relative effectiveness based on per cent of insects immediately killed by the spray. In the case of oil sprays used against the red scale, this criticism is especially justified because of the effect of the oil residue left by the spray on the surface of the tree, which impedes the successful establishment and development of the newly emerged, motile young progeny of those scales not killed by the spray. The greater the volatility and penetrability of an oil, the less is the inhibition of settling and development of the crawlers because of the reduced amount of oil remaining on the tree surface. An oil which is about 60 per cent distilled at 636° F (light-medium spray oil), with the addition of a toxicant, might result in as great a per cent kill of red scale as an oil about 35 per cent distilled at 636° (heavy-medium spray oil), without the addition of a toxicant, if both oils were used at the same per cent concentration in the spray. However, if two plots had identical scale population densities at the time of treatment and one were treated with the light-medium oil plus toxicant and the other with the heavy-medium oil, the latter would have the fewer scales per tree 6 months or a year later, although the initial kills were equal in the two plots.

The inhibitory effect of kerosene is probably of no practical importance, because on a warm day the kerosene will disappear from the tree surface within a few hours by penetration and evaporation. This has prompted an investigation as to the long-term effectiveness of kerosene plus toxicant as compared with regular spray oils even though the kerosene should result in a higher degree of initial effectiveness as based on per cent kill.

Observations in a Heavily Infested Lemon Orchard.—A lemon orchard in Covina, California, heavily infested with red scale, was selected for an experi-

[12] Data on file at the University of California Citrus Experiment Station, Riverside, California.

ment to determine the relative effectiveness, at long periods after application, of treatments which differ greatly in their ability directly to kill the red scale. Thirteen randomized plots, each with three subplots, were sprayed with various oils and kerosene with and without toxicants. Among these plots were the following: plot 1, 10 per cent kerosene-toxicant with 1 quart of kerosene-Cardolite-derris-extractives stock solution (see footnote 11) to 9¾ gallons of kerosene, toxicant A; plot 2, 10 per cent kerosene-toxicant with 1 pint of the

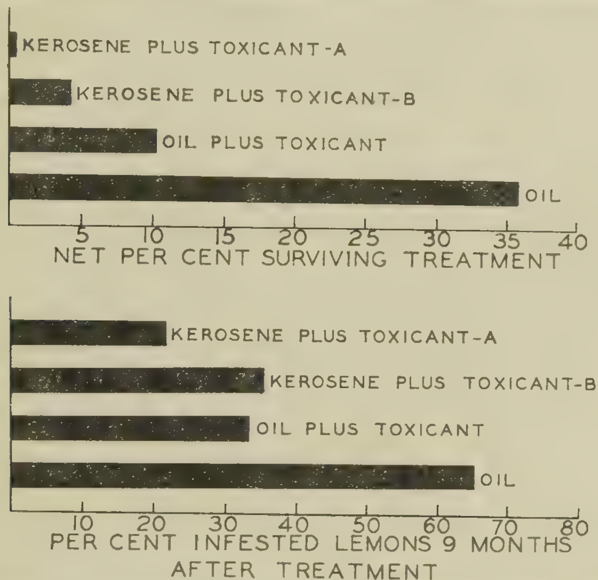

Fig. 2.—Relative effectiveness of four different treatments against the red scale in a heavily infested lemon orchard as shown by net per cent kill of adult female red scales on the branches of the trees a month after treatment (upper) and per cent of fruits found to be infested with red scale 9 months after treatment (lower).

toxic stock solution to 9⅞ gallons of kerosene, toxicant B; plot 3, 2 per cent light-medium oil with ½ pint of oil-Cardolite-extractives stock solution to 1$^{15}/_{16}$ gallons of oil; and plot 4, 2 per cent light-medium oil. Four ounces of calcium caseinate spreader to 100 gallons of spray was used in each plot.

The results of the experiment are shown in figure 2, where is depicted the relative effectiveness of the four treatments as based on per cent kill immediately after treatment and per cent of infested fruit on the trees 9 months after treatment. The treatments were applied November 18, 1941, and examinations to determine the per cent kill were made a month later. Then on September 1, 1942, four subplots, representing four different treatments which happened to be closely grouped in an area of the orchard in which the original population was reasonably uniform, were compared for relative infestation. All lemons less than 10 feet above the ground, on each of 10 trees in each subplot, which were more than half grown (about 1 inch in diameter) were examined and recorded as to whether they were free of red scales or infested. All the lemons which could be examined from the ground were counted, and simultaneously the number infested was determined.

The net percentages of adult females surviving the spray treatments (as based on counts made a month after treatment) in each plot were as follows: plot 1, 0.45; plot 2, 4.5; plot 3, 10.2; and plot 4, 36.1. These percentages represent the average of three subplots. The percentages of infested fruit on the trees 9 months after treatment, as determined by observations of four adjacent subplots, were as follows: plot 1, 22.4; plot 2, 34.9; plot 3, 31.6; and plot 4, 64.9. Of the net percentages of survival, all were significantly different from one another according to an analysis of variance; of the percentages of infested fruits 9 months later, all were significantly different from one another except that plot 3 was not significantly different from plot 2.

It should be borne in mind in the evaluation of the data presented in figure 2 that the relative lengths of the bars in the lower part of the figure show only the relative percentages of infested fruits, not the relative population densities. The plots having the greater percentages of infested fruits also appeared to have, from general observation, a greater average number of scales per fruit.

It is to be expected that, with passing time, differences in population density immediately following spraying would become increasingly smaller, resulting eventually in a complete obliteration of differences when all fruits on the tree become infested. In commercial procedure, of course, the orchard is usually treated before such a degree of infestation prevails. The rapidity with which the differences between plots are evened out depends on the original population density and the percentages of scales surviving the treatments, for the greater the number of scales in the plots following treatment, the sooner a condition will be reached wherein practically all fruits are infested.

It will be noted in figure 2 that although in the plot sprayed with spray oil plus toxicant (treatment 3) the net per cent survival was over twice as high as in one of the plots sprayed with kerosene plus toxicant (treatment 2), there was no significant difference in the per cent of infested fruit in the two plots 9 months after treatment. Thus the residual effect on the crawlers emerging from the scales not killed by the oil-toxicant spray offset the lesser immediate insecticidal effectiveness of the oil plus toxicant as compared with the kerosene plus toxicant. However, the plot sprayed with 10 per cent kerosene-toxicant with 1 quart of toxic solution to 9¾ gallons of kerosene (treatment 1), which resulted in the best kill of scales, was also the least heavily infested of all plots 9 months after treatment. This shows that a sufficiently high degree of initial kill with a material not having a long-term residual effect can overbalance in importance the advantage due to the residual effect possessed by less volatile oils.

Observations in a Heavily Infested Orange Orchard.—On October 21, 1942, an attempt was made to evaluate the relative effectiveness of some of the red-scale treatments applied to the heavily infested orange orchard sprayed April 9, 1942, the initial results of which, in terms of the percentages of insects surviving the treatment, were summarized in table 1 (orchard A, treatments 1 and 3) and table 3 (treatments 1 and 2). As can be seen from the tables, the per cent survival immediately after treatment was as follows: for the oil alone, 25.1; oil plus toxicant, 5.6; kerosene alone, 49.9; kerosene plus toxicant, 1.8. The examination of the plots in October revealed that all fruits on the trees sprayed with oil alone or kerosene alone were infested, but so were nearly

all the fruits on the trees sprayed with oil-toxicant or kerosene-toxicant. It was obvious, however, that the fruits in the plots sprayed with oil alone and kerosene alone were on the average more heavily infested than the fruits in the plots sprayed with oil-toxicant or kerosene-toxicant. Therefore, a determination of the number of scales per fruit would have been necessary to indicate the relative degree of infestation.

A random sampling of leaves, however extensively made, would not afford an accurate criterion of relative population densities because the leaves, after they are heavily infested, turn yellowish and finally drop to the ground. In October the leaves in the oil-sprayed plot had begun to drop to an appreciable extent, while in the kerosene-sprayed plot severe defoliation had already taken place. No abnormal defoliation occurred in plots in which toxicants were added to the spray. Thus, 6½ months after treatment, a general observation of the plots afforded a more adequate means of evaluating the long-term effectiveness of the treatments than any practicable statistical evaluation.

It will be noted that even those treatments resulting in the highest degree of control were not sufficiently effective to keep the fruit from being infested, and consequently made unmarketable, 6½ months after the treatments were applied. It must be borne in mind, however, that the orange orchard in question was extremely heavily infested at the time of spraying, and even a 99 per cent kill in such an orchard leaves large numbers of scales to reproduce in the tree. In an orchard of average infestation, it is common knowledge, an effective treatment may result in scale-free fruit even a year after treatment, while a materially less effective treatment in the same orchard, applied at the same time, may result in such a rapid reinfestation of the orchard that within a year the marketing value of the fruit may be greatly reduced.

DISCUSSION

Although the contributions of McBain and associates (1940, 1941, 1942) are most obviously related to the large and important fields of detergency and dyeing, their penetrating analysis of the theoretical implications of solubilization, as contrasted with ordinary molecular solution enhanced by the addition of hydrotropic or oleotropic solvents, can contribute much to a better understanding of the problems confronting oil-spray-research workers who are attempting to add oil-insoluble toxicants to oil.

The solubilizers are as a group relatively complicated, long-chain compounds as compared to the simpler oleotropic solvents such as *n*-butyl phthalate and diamyl phenol. Thus, for example, Cardolite 627 has a branched unsaturated chain of 14 carbon atoms attached *meta* to the –OH group of phenol. Solubilizing detergents are of similar complexity. In fact, McBain and Merrill (1942) believe that the effectiveness of a compound as a solubilizer increases with chain length to a limit fixed only by the necessity for having the solubilizer itself in solution. This difference in the chemical nature of the solvents may be used, at least roughly, as a guide in the selection of mutual solvents, according to the particular goal of the investigator.

Judging from the experience the present writers have had, true solubilization, despite its extreme efficiency in bringing about a visually clear and stable colloidal solution of derris or cubé extractives in the oil, should be avoided in

the preparation of oil-toxicants except when low concentrations of the extractives are adequate to bring about the desired insecticidal effect, as with the kerosene sprays of the present investigation. The possibility is not excluded, however, that further research may change or modify this belief.

It may be concluded, from present knowledge of the nature of solubilization, that insecticidal effectiveness, presuming identical concentrations of extractives in the oil-toxicant solution, is probably proportional to the true molecular concentration in the solution; this is probably greater when oleotropic solvents are used as mutual solvents than when solubilizers are used, for even though a larger amount of extractives is brought into solution by the latter, most of it is in colloidal solution and not in true molecular solution in the continuous liquid. Furthermore, from one fourth to one third of the rotenone-plus-deguelin content of an oil-toxicant having Cardolite as a solubilizer was found to pass from the oil to the water phase of an oil-water mixture agitated for 45 minutes.

Presumably the molecules of extractives bound within the colloidal micelles of a colloidal solution effected by means of a solubilizer are not so available for effect on the insect tissues as they would be if they were in molecular solution. As far as our present information indicates, however, molecular solutions of derris or cubé extractives in oil can be made only by (1) dissolving powdered extractives, or soaking the finely ground root of derris or cubé, directly in the oil; or (2) by using oleotropic solvents, which, as they are added, progressively increase the solubility of the extractives in the oil until in large quantities they make possible an adequate concentration of the extractives in molecular solution in the oil, besides effecting a stable suspension of a large percentage of the remaining extractives in extremely small particles, which, however, are not bound within colloidal micelles.

It is possible that the most effective mutual solvents, from the insecticidal standpoint, might leave a portion of the extractives in suspension when added to oil in the proportions which would appear to be practical either from an economic standpoint or from the standpoint of their effect on fruit or foliage.

The use of Cardolite as a solubilizer, as has been stated before, was first encouraged by the fact that visually clear and stable solutions of extractives could be formed in the oil, whereas with other solvents a certain cloudiness occurred even when exorbitantly large quantities of the solvents were used. In the latter case a completely ready-for-use oil-toxicant solution could not be manufactured because of the settling of the suspended material in the drums in which the oil was stored, which, if nothing else, resulted in a gummy residue in the drums which ruined them for further use. Kagy and Boyce (1941) have shown that a certain amount of precipitation does not necessarily indicate a reduction in toxicity because the toxic ingredients may remain in solution in the oil.

From the standpoint that stable solutions are more amenable to proprietary exploitation than partial suspensions, the solubilizers lend themselves more readily to commercial use than oleotropic solvents. Trials with Cardolite as a solubilizer for incorporating derris extractives into the heavier spray oils, however, showed inconsistency in results. In no case were results so good as might be expected on the basis of earlier work with the same amounts of oil

and extractives, but with *n*-butyl phthalate as a mutual solvent. The data presented in this paper further illustrate the superiority of the oleotropic solvents.

If sufficient advantage in the way of insecticidal effectiveness is found in the use of oleotropic solvents, it might, apparently, be advantageous to prepare the mutual solvent-extractives solution separately from the oil and add it to the oil immediately before use. However, it is possible that a way may be found to use Cardolite, or other solubilizer, in spray oil in a manner that will permit a more efficient utilization of the solubilized extractives at the higher concentrations.

It has been shown in this paper that a high concentration of rotenone plus deguelin in light-medium spray oil may be obtained merely by soaking finely ground cubé root in the oil for 20 minutes, while stirring vigorously. The advantages of this method of dissolving the extractives in oil are that (1) the extracted toxic constituents of the root are entirely in true molecular solution, in which condition they have their greatest possible insecticidal value; (2) the danger of foliage injury or reduction in oil deposit which may be caused by a mutual solvent is eliminated; and (3) the method is the most economical of all known means of incorporating derris or cubé extractives into spray oil. Cubé extractives, however, are more easily extracted from the ground root than are derris extractives.

SUMMARY

Powdered extractives of rotenone-bearing plants may be dissolved directly in spray oil at room temperature in concentrations of insecticidal value by mixing the powder in oil for a 20-minute period. In the same manner the extractives may be obtained from finely divided plant material. In either case an emulsive oil was found to be a better solvent for the extractives than straight oil of the same grade; and to obtain the highest concentrations in oil, mutual solvents must be used.

A *solubilizer* is a mutual solvent which in very dilute concentration, usually 1 per cent or less, will produce a thermodynamically stable colloidal aqueous or nonaqueous solution of otherwise insoluble or only slightly soluble substances.

The term *oleotropic solvent* is proposed for mutual solvents which, when added to an oil in *large amounts*, will increase the solubility of an otherwise insoluble or slightly soluble substance in oil merely by adding their solvent properties to that of the oil.

No rotenone plus deguelin was found to have passed from the oil to the water phase of an oil-water mixture agitated for 45 minutes when the extractives were in true solution in the oil. However, under the same conditions a colloidal solution of extractives in oil may lose from one fourth to one third of its rotenone-plus-deguelin content in an oil-water mixture agitated for 45 minutes.

Cardolite 627 is a highly efficient solubilizer for incorporating derris extractives in spray oil, forming a visually clear, stable solution which is probably mainly colloidal; but *n*-butyl phthalate, as an example of a good oleotropic solvent, will form a slightly cloudy solution when 5 per cent derris extractives

in *n*-butyl phthalate is added to spray oil at the rate of 1 part to 7 parts of oil. Nevertheless an oil spray with an optimum concentration of derris extractives brought into partial solution in the oil by means of *n*-butyl phthalate as a mutual solvent is far more effective against the California red scale than an oil spray used at the same strength and containing the same concentration of derris extractives, but having Cardolite 627 as a solubilizer.

The increase in the insecticidal effectiveness of the oil due to the addition of a solution of *n*-butyl phthalate extractives is brought about despite the reduced amount of oil deposited on the tree surface because of the emulsifying effect of the mutual solvent.

It is assumed that the greater insecticidal effectiveness of the oil-toxicant solutions containing oleotropic solvents, as compared with those containing solubilizers, results from the fact that the molecules of extractives are mainly in molecular solution; in this condition they are probably more effective against insect tissue than when they are bound within the lamellar micelles or otherwise constituted colloidal particles resulting from solubilization. Likewise, as stated before, a substantial proportion of the colloidal particles will pass from the oil to the water phase of an agitated spray mixture if the extractives are in the oil in the form of a colloidal solution.

Freshly prepared kerosene-Cardolite-extractives solutions are highly effective against the red scale when added to kerosene to make a 0.103 per cent concentration of total extractives (0.031 per cent concentration of rotenone) in the kerosene. A 6-weeks' period was sufficient to cause considerable decomposition of the toxic solution in which Cardolite was used as a solubilizer. This decomposition can be minimized by the addition of an antioxidant.

An adequate concentration of derris or cubé extractives can be obtained merely by soaking ½ pound of finely ground derris or cubé root in 10 gallons of kerosene for 20 minutes. This results in a concentration of rotenone plus deguelin in the kerosene of 0.05 grams per 100 ml. The finely ground root also increases the effectiveness of regular oil spray when added at the rate of 4 ounces to 1 gallon of regular spray oil, but in the field work reported in this paper the ground root has not been stirred in the oil for a sufficiently long period to result in the maximum concentration of rotenone plus deguelin in the oil. A continuous stirring of ground cubé root in emulsive spray oil for 20 minutes will result in the maximum degree of extraction of the toxic ingredients from the root particles, and, if such a procedure were practiced, it is probable that the ground root soaked directly in the oil would afford the most effective practicable means of using this product with spray oil against citrus pests. The toxic ingredients of derris root are not so readily extracted by spray oil as those of cubé root.

When added to spray oil, the rotenone-free extractives (deguelin concentrate) of derris appear to be about as effective as rotenone when identical concentrations of each are used. However, the complete extractives are more effective than equal concentrations of either the rotenone or the rotenone-free extractives used by themselves; evidently the latter two are synergistic. When added to kerosene the rotenone-free extractives were not so effective as the rotenone, even though the latter was of necessity used at a lower concen-

tration; neither were they so effective as the total extractives when used at the same concentration.

It may be that the best mutual solvents, from the standpoint of insecticidal effectiveness, will not lend themselves to the preparation of finished, ready-for-use spray oils with toxicants because a certain percentage of solids may precipitate upon standing. If this be the case, it is suggested that the mutual solvents and extractives be prepared in a separate solution to be added to the oil just before the oil is poured into the spray tank.

An oil film on the tree, impeding the settling and development of "crawlers" issuing from scales not killed by an oil spray, was demonstrated to influence markedly the effectiveness of the treatment 9 months after spraying when the oil-toxicant spray was compared with a kerosene-toxicant spray, which leaves no oily residue because of rapid penetration and evaporation of the kerosene. However, when the initial per cent of kill from a kerosene-toxicant spray was sufficiently high (99.5 per cent) it more than offset the disadvantage of lack of oily residue, and resulted after a 9-month interval in trees more free of scaly lemons than those sprayed with the regular light-medium spray oil with or without a toxicant.

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